Effect of Leucas aspera (Willd.) Spr. and Monochoria vaginalis (Burm.f.)C.Presl. in rat model of hepatotoxicity

SYNOPSIS

Liver disease is a worldwide problem. Herbal drugs are playing an important role in the treatment of liver diseases mainly due to the general belief that they are without any side effects, besides being cheap and locally available. Till date there is no effective medicine for hepatic disorders, such as hepatitis. Consequently control of liver diseases has become a major goal of modern medicine. As it is the function of hepatoprotective agents to interfere with these pathological processes by blocking their evolution and helping recovery, the development of new antihepatotoxic drugs is the need of the hour.

Carbon tetrachloride (CCl_4) intoxication is an experimental model worldwidely used to study hepatic disorders. CCl_4 produces an experimental hepatotoxicity which histologically resembles viral hepatitis. Hence, any drug devised for the treatment of CCl_4 induced hepatopathy may surely prove effective in combating liver disorders.

Most of the hepatoprotective drugs now available in the market cost much, and hence there is a genuine need to devise some cost effective drugs based on plant principles. Though we know the hepatoprotective potential of many plants, there is still paucity of information regarding the potential of *Leucas aspera* and *Monochoria vaginalis* in treating hepatic disorders. Hence in this study two plants *Leucas aspera* and *Monochoria vaginalis* were selected to validate their traditional claim. *Leucas aspera* commonly known as Thumbe, belongs to the family Lamiaceae. Certain reports indicate the liver protective efficacy of this plant. But no detailed investigation has yet been carried out in this respect. So in this study the whole plant is subjected to phytochemical and pharmacological analysis with special emphasis on hepatoprotection. *Monochoria vaginalis* commonly known as Karinkuvvalam in Malayalam, is a plant belonging to the Pontederiaceae family known to have several

medicinal properties. It is a weed found in rice fields and in some parts of India it is used as a vegetable. Roots of *Monochoria vaginalis* is used in traditional medicine for the treatment of hepatopathy. So the main objective of the study is to evaluate the antioxidant and hepatoprotective potential of *Leucas aspera* and *Monochoria vaginalis* in experimental models.

The first chapter of the thesis is an introduction, including a review of literature on hepatoprotection, liver fibrosis, liver cancer, liver protective medicinal plants etc. The second chapter is the method section, discussing the various experimental approaches and the techniques used in this study mainly in vitro antioxidant analysis, biochemical analysis, histopathological analysis, immunohistochemical analysis, LC-MS analysis, MTT assay, fluorimetric analysis, flow cytometry etc. The results obtained are described in the following sections.

The first part of the result section is preliminary phytochemical screening and in vitro antioxidant analysis. Preliminary phytochemical analysis of different extracts of *Leucas aspera* and *Monochoria vaginalis* revealed the presence of flavonoids, alkaloids, phenols, tannins, carbohydrates and proteins in the methanolic extract. Also, the methanolic extract of *Leucas aspera* (MELA) and *Monochoria vaginalis* (MEMV) exhibited the highest free radical scavenging activity. Hence the methanolic extract of both plants were selected for further in vivo studies.

The methanolic extract of L. aspera (MEMV) was subjected to further sub fractionation using petroleum ether, ethyl acetate, chloroform and methanol. The IC_{50} of the different sub fractions were found out. The Petroleum ether fraction showed lowest IC_{50} having good antioxidant activity. So it was subjected to LC-MS analysis. LC-MS analysis of petroleum ether fraction of MELA revealed the presence of five major phytochemicals with proven

antioxidant/anticancer properties viz. beta-sitosterol, chrysoeriol, dotriacontanol, leucasperone A and leucasperone B.

Based on this background, a dose depended study was conducted in male wistar rats to prove the in vivo antioxidant and hepatoprotective potential of the two plants. Both preventive and curative effects of these two plants were tested in CCl_4 intoxicated rats. Both the extracts of MELA and MEMV significantly ($p \leq 0.05$) prevented and reversed the elevation of serum AST, ALT, ALP and tissue TBARS levels in both the experimental models. Also hepatic GSH, GST, GPx, Catalase and SOD were remarkably increased by the treatment with the two extracts. Histopathological changes also supported the preventive and curative effects of the extracts and the results were comparable with silymarin, the standard hepatoprotective drug. Further, in both the experimental models, the extract exhibited its antioxidant potential in a dose dependent manner.

In view of this a detailed study was carried out to test the antifibrotic efficacy of these two plants. Liver fibrosis was induced in male Wistar rats by the administration of carbon tetrachloride (150 µl/100 gm; oral) for 10 weeks. In pre-treatment groups plant extract treatment started one week before the onset of CCl_4 administration and continued up to 10 weeks. In curative treatment groups extract at 100 and 200 mg/kg were administered for two weeks after 10 week of CCl_4 induction. Both the extracts significantly ($p \leq 0.05$) prevented or reduced an increase in serum AST, ALT, ALP activities, tissue lipid peroxides and hydroxyproline levels in CCl_4 intoxicated animals in both treatment groups. Also, the extract treatment increased liver GSH levels and the serum levels of total protein and albumin in a dose depended manner.

Histopathological analysis of liver tissues further corroborated the biochemical findings. Immunohistochemical analysis of liver sections specifically stained for collagen-III showed that collagen-III was predominantly expressed along fibrous septa in CCl_4 treated rats.

Weak immunostaining of localized collagen - III was noticed in MELA and MEMV extract treated rats, at doses of 100 mg/kg and 200 mg/kg body weight co-exposed with CCl_4. Weak immunostaining of localized collagen III was observed in the standard drug silymarin treated rats.

LC-MS analysis of petroleum ether fraction of MELA revealed the presence of five major phytochemicals with proven antioxidant/anticancer properties. So another study was conducted to test the anticancerous efficacy of MELA against N-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma in male rats. In this study an increased level of alpha fetoprotein (AFP) and carcino embryonic antigen (CEA) were observed in NDEA treated rats, confirming the presence of hepatocellular carcinoma. Elevated levels of serum AST, ALP, LDH, γ-GT, bilirubin and lipid peroxides were also observed in the NDEA treated rats in both models. The result showed that hepatocellular carcinoma induced by *N*-nitrosodiethylamine was effectively inhibited by the treatment with *L.aspera ex*tract at a dose of 200 mg/kg in both preventive and curative models.

The cytotoxic activity of *L.aspera* (100-1000 μg/ml) against HeLa cells was assessed in vitro by MTT proliferation assay. 0ne hundred to 1000 μg/ml of *L.aspera* significantly reduced the viability of HeLa cells. DNA-binding dyes acridine orange and ethidium bromide were used for the morphological detection of apoptotic and necrotic cells. LDH leakage assay was performed with cell free supernatant collected from tissue culture plates. Monitoring a cell's ability to proliferate is critical for assessing a cell's health during toxicity studies by directly measuring DNA synthesis by flow cytometry. Flow cytometric analysis of HeLa cells treated with methanolic extract showed significant inhibition of cells at Go/G1 phase.

Examination of in vitro cytotoxicity revealed that the extract might be a significant source of potential anticancer compounds in view of their pronounced cytotoxic activities against HeLa cells. Morphological analysis of the mode of HeLa cell death, together with the cell cycle

analysis showed that the treatment of Hela cells with higher concentrations of the extract induced apoptotic cell death. Due to their ability to promote apoptotic cell death in cancer cells, the extract and their constituents may have significant anticancer potential.

Thus the present study on *Monochoria vaginalis* conclude that the plant was found to be highly effective in resisting hepatic fibrosis. The antioxidant and antifibrotic properties of the extract may be due to the combined effect of alkaloids, phenols, glycosides, tannins and flavonoids present in the root. Future studies may be carried out to isolate the active principle responsible for the hepatoprotective action. Also, the protective mechanism of action of the active principle can be explored.

Leucas aspera possess excellent antioxidant, antifibrotic and anticancer properties. These medicinal properties of the plant may be due to the combined activity of the identified phytochemicals such as beta-sitosterol, chrysoeriol, dotriacontanol, leucasperone B and leucasperone A in the active fraction of MELA. It is possible that the anticancer effect of the extract is mediated through antioxidant and / or free radical scavenging activities. This investigation further demonstrates that *L.aspera* extract is a promising chemopreventive agent for cancer and might be useful clinically after further molecular chemopreventive studies.

The isolation of active phytochemical constituent from *L.aspera* and the determination of their individual antioxidant /hepatoprotective activity will be further analysed at molecular level. The immunomodulatory properties of the active fraction can also be studied.